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Genetic regulation of the specific and non-specific component of immunity

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1. Summary

Bi-directional selective breeding for antibody (Ab) responsiveness to heterologous erythrocytes (Selection I) produced a high (H) and a low (L) responder line of mice which were also remarkably separated for Ab responses to many unrelated natural antigens (Ags) such as heterologous proteins, viruses, bacteria, parasites and haptens carried by these immunogens. The character "quantitative Ab responsiveness" is controlled by several independently segregating loci (polygenic regulation). The major genetic modification is produced at the level of macrophage activities. The Ag is slowly catabolized and persists for a long time on the macrophage membrane of the H line, whereas it is rapidly destroyed in L line macrophages. The bactericidal and bacteriostatic activity of the macrophage is also strong in the L line and weak in the H line. The opposite genetic regulation of Ab responsiveness and macrophage activity is a fundamental phenomenon for understanding natural and vaccination-induced anti-infectious immunity. The L line is more resistant than the H line against the infections due to intracellular microorganisms (*Salmonellae, Yersinia, Mycobacteria, Brucellae, Leishmania*) where the macrophage plays the dominant defensive barrier. The H line is more resistant than the L line to the extracellular microorganisms which are efficiently counteracted by a strong antibody response (Pneumococcus, Klebsiella, Plasmodia, Trypanosoma). The intensity of T cell-mediated immunity as measured by delayed type hypersensitivity, which is independent of the genetic regulation of antibody responsiveness, is correlated with the degree of non-specific inflammation produced at the site of the reaction by the Ag injection in non-sensitized mice. The intensity of the non-specific inflammatory reactions to an inert substrate presents a continuous phenotypic variation in inbred lines of mice. Therefore this character is subject to polygenic regulation. The inflammatory reaction constitutes one of the most important non-specific components of immunity. We have therefore initiated a selective breeding experiment to produce lines of mice endowed with maximal and minimal intensity of non-specific inflammatory reactions. This model of selective breeding is described.

Key words: Genetic regulation; Antibody; Cellular immunity; Inflammation; Infection

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2. Introduction

In this article the term "immunity" is taken in its broadest meaning, including all the factors modifying the resistance or susceptibility of mammals to infectious diseases produced by viruses, bacteria, protozoal and metazoal parasites. We shall consider only the germ line transmitted factors which modify immunity, excluding all the environmental, nutritional, behavioral and local effects that may also

produce important modifications in immunity. The infectious agents tend to multiply in the susceptible host and produce toxic molecules, so causing the peculiar lesions characterizing each type of infection. The organism tends to counteract the infection by complex cellular and molecular mechanisms which limit the proliferation of the infectious agents and neutralize the toxic molecules produced.

The defense mechanisms may be classified into two broad categories: specific and non-specific. The specific component is operated by the immune system through its two fundamental mechanisms of antibody-mediated and T cell-mediated immunity. The principal characteristic of the specific immunity is a long-lasting memory that regulates the speed and intensity of secondary responsiveness. The non-specific component relies on stable cellular and molecular mechanisms including: phagocytic cells, complement system, blood coagulation system, and the cascade of interdependent phenomena constituting the inflammatory reaction. Of course, these two immunity components have large and complex interactions in particular at the level of the macrophages. These cells act as central regulatory cells of specific immunity [1], are affected by the products of immune reactions (opsonizing antibodies and interleukins) and also play a very important role in the mechanism of inflammation.

The presence or absence of specific memory is the fundamental characteristic differentiating the two components of immunity. It is the memory of past aggressions that renders the specific immunity a highly adaptive phenomenon, whereas the non-specific component is devoid of such property. Genetically heterogeneous natural animal populations adapt to the infectious agents of their environment. This is the principal phenomenon determining the innate immunity of the population in its ecological niche. The adaptive effect of the specific immunity memory constitutes the basis of the artificially increased immunity induced by prophylactic vaccination. This common property accounts for the positive correlation frequently observed between the genetic regulation of innate and vaccination-induced immunity [1, 2].

The bases of the genetic regulation of both specific and non-specific immunity have principally been established by experiments carried out in mice.

3. Genetic regulation of specific immunity

Our experiments of bidirectional selective breeding for antibody (Ab) responsiveness to complex natural antigens (Ags) have clearly demonstrated that this important parameter of specific immunity is regulated by the additive interaction of several independently segregating loci (polygenic regulation) [3]. A schematic representation of the results of a bidirectional selective breeding experiment for quantitative antibody responsiveness is reported in Fig. 1.

The assortative mating of highest and lowest phenotypes for serum antibody response, culled out from a genetic heterogeneous population, was repeated for the number of consecutive generations required to obtain the maximal interline separation (selection limit). The resulting high (H) and low (L) responder lines are characterized by a very large interline difference in Ab responsiveness and a small intraline variability. At selection limit, all the "high effect" alleles have segregated in H line and all the "low effect" alleles in L line which therefore become homozygous at the level of all the locus participating in the quantitative regulation of the character "Ab responsiveness". The homozygous H and L line phenotypes are largely outside the extreme phenotypes found in the distribution of the genetically heterogeneous population used as foundation population (F_0). The mean Ab responses of the inbred lines produced irrespective of quantitative antibody production have a high probability of being close to

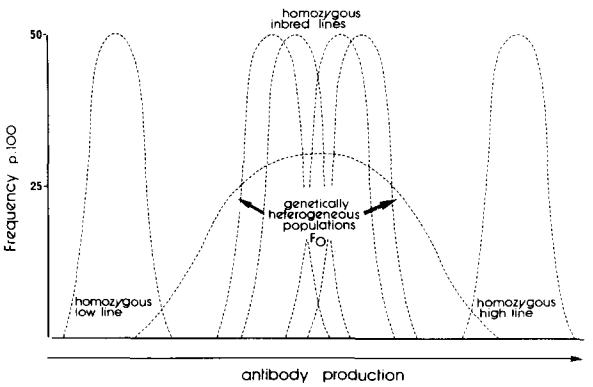


Fig. 1. Distribution of phenotypes for a character subject to polygenic regulation (antibody production) in outbred mice, inbred lines of mice and lines of mice obtained by bidirectional selective breeding (H and L lines) (From Biozzi et al., 1985 [4]).

the modal phenotype in the distribution of the natural F_0 population. The phenotypic variability (variance = V) of F_0 population is large since it results from the interaction of genetic (genetic V = VG) and environmental factors (environmental V = VE). The intraline variability in genetically homozygous inbred lines as well as in homozygous H and L lines is small and measures the VE for the character investigated.

It may be reasonably postulated that the interline difference in resistance or susceptibility to infections between H and L Ab responder lines is mainly due to specific immunological mechanisms, whereas that separating the inbred lines would principally rely on non-specific host – parasite interactions. For a more complete description see [4].

3.1. *Selective breeding for specific immune responsiveness*

Six independent selections were carried out for Ab responsiveness, using distinct Ags and different immunization procedures: Selection I, II, III, IV, IV A and V [2, 3] and two multi-antigen selections: GS [5, 6] and GP (data to be published). The character "quantitative antibody response to all the Ags" used in these selections is controlled by polygenic regulation. Another important common characteristic is the multi-specific effect of each selective breeding.

In the present study we shall only consider the results obtained in Selection I, the first made and the most extensively investigated [7].

3.1.1. *Modification of antibody responsiveness*

Selection I was performed for agglutinin response to intravenous immunization with sheep erythrocytes (SE) and pigeon erythrocytes (PE) alternated in consecutive generations to avoid the interference of maternally transmitted Ab [7]. The very large phenotypic effect produced in Selection I is illustrated in Fig. 2, which shows the kinetics of agglutinin responses to SE in H and L lines.

Intravenous immunization with the optimal dose of SE produced a very strong and long-lasting agglutinin response in the H line and only a weak and transient response in the L line (Fig. 2, A). These kinetics essentially reflect the spleen immunocyte contribution as demonstrated by splenectomy experiments [8]. The genetic modification of Ab responsiveness produced by the selective breeding is not limited to

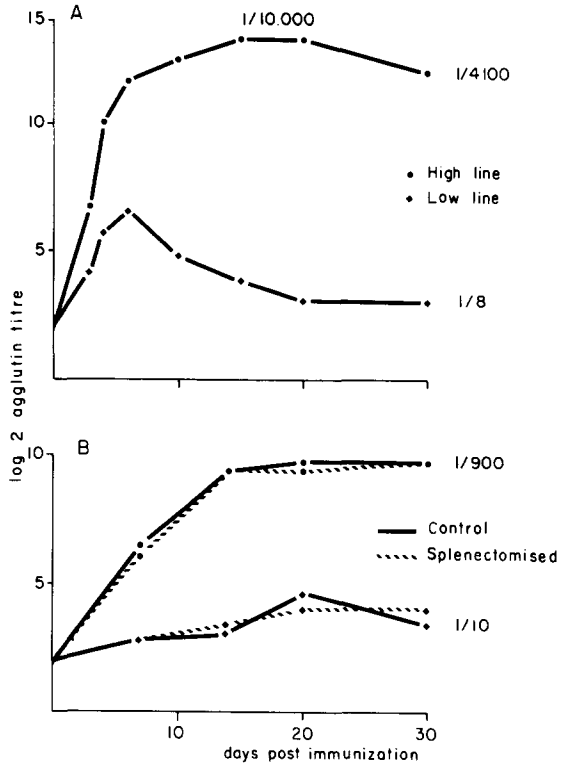


Fig. 2. Kinetics of agglutinin production to SE in H and L lines. A intravenous immunization with 5×10^8 SE. B Footpad immunization with 10^7 SE. Effect of splenectomy performed 24 h before SE injection.

the spleen but involves all the regions of the immune system. In fact, a small dose of SE injected in a hind footpad induces an Ab synthesis also much stronger in H than in L line (Fig. 2, B). In this experiment, the demonstration that the Ab production is strictly limited to the local lymph nodes is given by the total lack of effect of splenectomy performed 24 h before footpad immunization.

The large difference separating H and L lines is not restricted to the specificities of the selections Ags (SE and PE) but is also observed for a large number of Ag specificities, encompassing other heterologous erythrocytes, proteins, viruses, bacteria and parasite Ags, T-independent polysaccharides, transplantation Ags, synthetic polypeptides and haptens (multi-specific effect). This general multi-specific effect is extremely large in Selection I, which therefore is particularly suitable for the investigation of

the genetic modifications of anti-infectious specific immunity.

3.1.2. *Modification of macrophage activity*

Antigen metabolism and presentation An important modification of macrophage activity was produced in Selection I, demonstrating the central regulatory function of macrophages in Ab responsiveness. Macrophages are characterized by potent phagocytic activity and efficient phagosome enzyme equipment apt to digest the Ags and to kill living ingested microorganisms. Another important macrophage function is to display the digested Ag fragments on the membrane so giving the specific information to T and B lymphocytes. It is particularly interesting that these two fundamental macrophage functions have independent genetic regulation. In fact, the phagocytic activity of liver and spleen macrophages is similar in H and L lines, whereas the Ag metabolism and membrane display present a striking interline difference [1, 9, 10].

The speed of Ag catabolism is markedly higher in L than in H line macrophages. The half-life of immunogenic SE Ags in spleen macrophages is 12 h in L line and 54 h in H line. The continuous and long-lasting immunogenic stimulation provided by H line macrophages is principally responsible for the strong and protracted Ab response, whereas the shortage of immunogenic stimulation due to the rapid Ag catabolism in L line macrophages accounts for the weak and transient Ab response illustrated in Fig. 2, A.

The threshold immunogenic dose of SE is about 100-fold lower in H than in L mice [11]. This interline difference is even larger (10,000-fold) for bovine serum albumin [12]. A comparison of dose–response relationship shows that the SE dose producing an equivalent Ab response is 1000-fold larger in L than in H line mice.

In our early cytodynamics studies [13], we demonstrated that the sudden stop of the exponentially ascending phase which occurs around the 5th day after intravenous immunization is due to antigen shortage. H and L mice were therefore immunized with repeated Ag injections. The results in Fig. 3, A show that 4 and even 2 SE injections at short intervals greatly increase the antibody response in L mice. In contrast, in the H line similar responses are produced by 1, 2 or 4 SE injections. These results indicate that the poor Ab response of L line is due to a deficient antigenic stimulation which can be par-

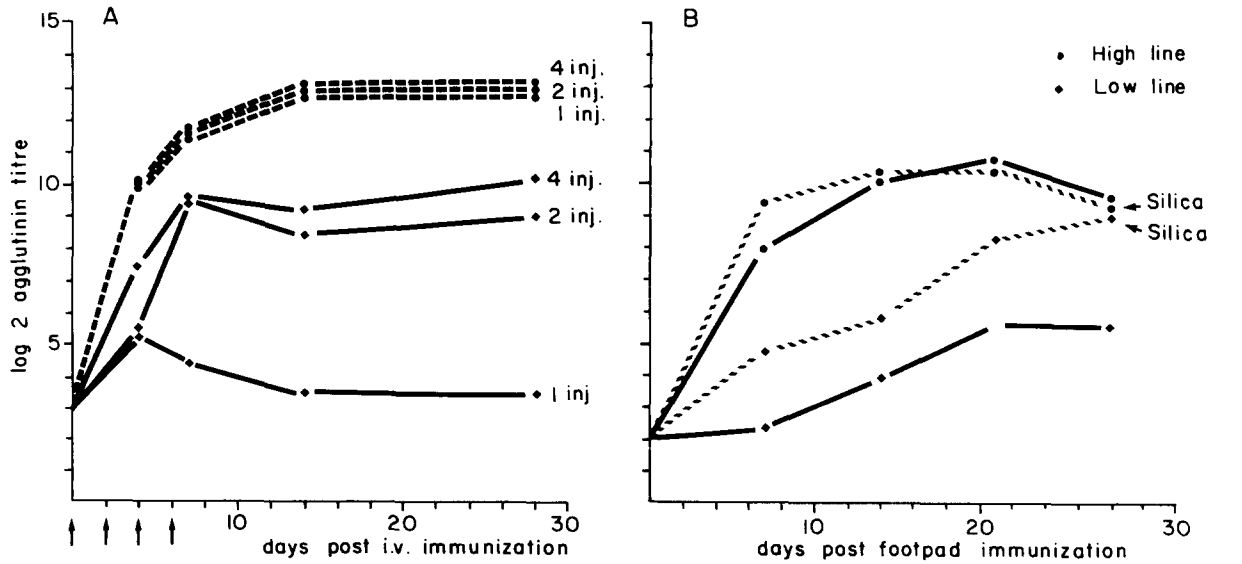
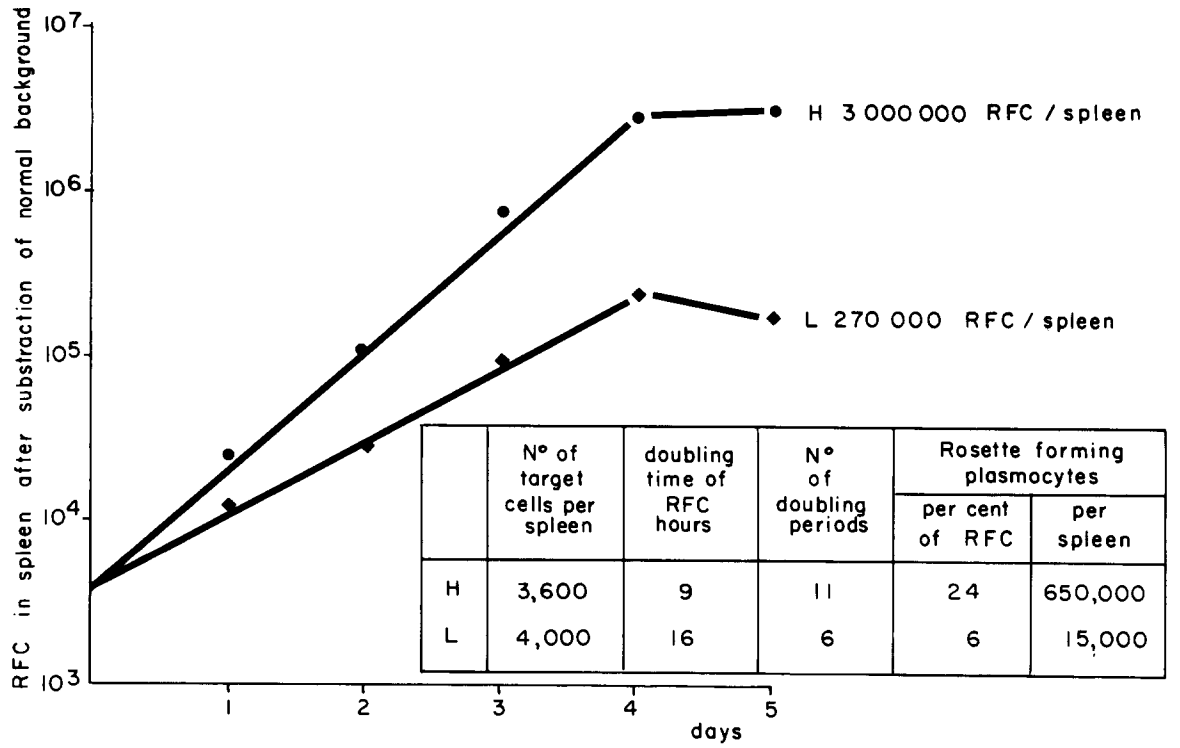


Fig. 3. Kinetics of agglutinin production to SE in H and L lines. [A] Response to single or repeated intravenous injections of 5×10^8 SE. [B] Effect of silica on response to local immunization: 5 mg of Silica given subcutaneously in one hind footpad 24 h before 10^7 SE injected at the same site.

tially compensated by Ag supplies. In the H line, on the contrary, a single injection induces an optimal antigenic stimulation [1]. This interpretation is confirmed by the results of experiments in which silica was used to decrease the macrophage catabolic activity on ingested SE Ags. When 5 mg of silica are injected in the footpad 24 h before the local immunization, a marked increase in Ab responsiveness, compared with control immunized mice, is produced in L line only [14] (Fig. 3, B). Another experimental approach demonstrating the important role of macrophages is based on the radiation resistance of Ag metabolism and presentation process. When H and L mice are immunosuppressed by irradiation, restored with an identical number of isolated spleen cells from (H × L)F_1 hybrids, and immunized, the antibody response of H line largely exceeds that of L line [15].

The slower Ag catabolism, accompanied by a higher Ag membrane concentration in H than in L line macrophages, produces important modifications in the cytodynamics of the specific responses illustrated in Fig. 4. In this experiment, the expansion of the specific clone of Ab forming cells in the spleen was measured by the rosette method [13]. H and L lines of mice of Selection I were immunized intravenously with an optimal dose of SE and the number and morphology of rosette forming cells (RFC) in the spleen were determined in the 7-day period of post immunization encompassing the exponential phase of the immune response. This experimental model permits the determination of the principal cytodynamic parameters characterizing the exponential rise of the response. Starting from a similar number of Ag sensitive target cells extrapolated at 0 time, the expansion of the specific immunocyte clone occurs much faster in H than in L line. The RFC doubling time in H line is about half of that in the L line, therefore the exponential phase encompasses 11 doubling periods in the former and only 6 in the latter line, producing a more than 10-fold interline difference in the number of RFC at the end of the exponential period lasting about 100 h. The study of the morphology of RFC in



	N° of target cells per spleen	doubling time of RFC hours	N° of doubling periods	Rosette forming plasmocytes	
				per cent of RFC	per spleen
H	3,600	9	11	24	650,000
L	4,000	16	6	6	15,000

Fig. 4. Cytodynamic parameters of the immune response measured by the splenic rosette forming cells (RFC) in H and L mice immunized with 5 × 10^8 SE i.v. (from Biozzi et al., 1972 [11]).

stained preparation permits the assessment of the rate of differentiation of RFC, starting from the small lymphocytes which constitute the totality of Ag-sensitive target cells, up to the completely differentiated Ab-secreting plasmocytes. At the end of the exponential phase, the number of RF plasmocytes in the spleen is more than 40-fold higher in H than in L mice. This interline difference is in agreement with that of the serum agglutinin titers on the same day [11]. The results of these experiments demonstrate that the genetic modification of Ag handling in macrophages regulates the Ab responsiveness by modifying the rate of multiplication and differentiation of the specific immunocyte clone. This process, which is independent of the Ag specificity, accounts for the general multi-specific effect of the selective breeding.

Bactericidal and bacteriostatic activity A very important macrophage function concerning anti-infectious immunity is the control of the survival of the phagocytized living microorganisms. Non-pathogenic or low virulent microorganisms are killed inside macrophages (bactericidal effect), whereas the rate of intracellular multiplication of virulent pathogens is reduced (bacteriostatic effect). It is very probable that the Ag metabolism and the intracellular microorganism survival depend on the potency of the enzyme equipment of macrophage phagosomes, since both phenomena are modified in the same direction by the selective breeding. In fact, the non-pathogenic T_4 bacteriophage is inactivated much faster in L than in H line macrophages [16]. A stronger bacteriostatic effect of L mice macrophages was also observed on several pathogens such as *Salmonellae* [17], *Brucellae* [18], *Mycobacterium tuberculosis* [19], *Yersinia* [20], *Leishmania* [21] and *Chlamydia* [22]. Comparative assay on the bactericidal and bacteriostatic activity of H and L line spleen macrophages using temperature sensitive mutants of *Escherichia coli* and *Salmonella typhimurium* shows that the slower growth of phagocytized virulent microorganisms in L line macrophages is due to a stronger bacteriostatic effect rather than to an increased bactericidal activity [1].

3.1.3. *Cell-mediated immunity*

The delayed type hypersensitivity (DTH) reaction is the T-mediated response most commonly measured in mice. The intensity of DTH reaction was measured by the increase of ear thickness 24 h after the local application of picryl chloride in mice sensitized 6–7 days previously by skin painting. The DTH reaction was hardly significantly larger in H than in L mice. However, at the same time a humoral response also developed, about 7-fold stronger in H than in L mice. Therefore the local ear infiltration is not the expression of a pure DTH reaction [23].

A better DTH measurement was made by Milon and Marchal who counted the number of specific T cells mediating DTH in the spleen of SE sensitized H and L mice, using an original local adoptive limiting dilution transfer test [24]. They have demonstrated that the number of specific T cells in the spleen of immunized mice is markedly higher in H than in L mice. Moreover, the comparison of dose–response relationship shows that L line requires a 1000-fold larger sensitizing dose of SE than H line to produce an equivalent number of specific T cells [25]. This interline difference agrees perfectly with that concerning Ab responsiveness to SE mentioned above. The number of SE-specific T cells present in the spleen before sensitization is similar in H and L mice; therefore, the expansion of the specific T lymphocyte clone mediating DTH in sensitized mice is much larger in H than in L line, just as for the clone of antibody producing B lymphocytes. This interline difference of T cell-mediated immunity responses is due to the macrophage modifications concerning Ag metabolism and presentation described previously. In fact, the number of SE-specific T cells in the spleen of lethally irradiated H and L mice restored with an equivalent number of spleen cells isolated from $(H \times L)F_1$ donors and sensitized with SE is 25-fold larger in H than L line recipients [25].

Moreover, repeated SE injections preferentially increase the number of SE specific T cells in the spleen of L mice, just as has been demonstrated for Ab responses (see Fig. 3, A). The T cells detected by their ability to mediate DTH reactions are T helper lymphocytes [26]. This finding agrees with our observation that the dose of anti-T helper (L_3T_4) monoclonal Ab required to inhibit 50% of in vivo Ab response to SE is 40-fold higher in H line (Mouton et al., data to be published).

All the findings described in Section 3.1.2. converge to the general conclusion that the central

regulatory role of macrophages on both specific B and T cell responses in H and L lines is due to genetic modifications of Ag metabolism and presentation.

3.1.4. *Lack of correlation between the specific responses and the final phenotypic expression of T cell-mediated immunity*

Comparing the above-reported results of Milon and Marchal [25] with those obtained in our laboratory, it is clear that the intensity of DTH reaction measured by the increase of footpad thickness is independent of the number of specific T cell mediators of DTH reactivity.

The results in Table 1 show that the specific T cell number and Ab responses on the 13th day correlate with the genetic status of the mice. The lack of correlation with the final expression of the footpad reaction indicates that other limiting factors operate on the local inflammatory reaction.

Similar findings in H and L lines of Selection I have also been observed for other manifestations of cellular immunity such as graft versus host reaction (GVH) and allogeneic skin graft rejection. The intensity of GVH reaction measured by spleen weight increase (spleen index) in new born $(H \times DBA)F_1$ and $(L \times DBA)F_1$ hybrids injected with equivalent numbers of spleen cells from adult H and L donors was identical [27]. The rejection time of skin allografts was similar in H and L lines (12.6 ± 2.9 and 10.4 ± 2.4 days respectively ($P < 0.1$)) but the H-2 cytotoxic serum antibody titers measured on skin donor lymphocytes after skin graft rejection was 1/110 in H and 1/3 in L skin graft recipients [28].

The intensity of these three T cell-mediated reactions: DTH, GVH, and allograft rejection, does not depend on the number of specific T cells but is determined by the local infiltration of a majority of non-specific leukocytes attracted locally by the lymphokine specifically released by a minority of sensitized T cells. It is essentially the intensity of the non-specific inflammatory reaction which limits the final expression of the T cell-mediated immunity reactions. In order to verify that the final expression of DTH is always independent of the genetically determined Ab responsiveness, we repeated the experiments of Table 1 in the 6 Selections for Ab responsiveness carried out in our laboratory: Selections I, II, III, IV, V and GP [29]. The results of this experiment, reported in Fig. 5, show a continuous progressive increase in the intensity of DTH reactions in the different lines of mice. There is no correlation at all between the H and L Ab responsiveness status of each line and the intensity of DTH reaction. The genetic drift occurring in each closed colony of selective breeding for Ab responsiveness produced a high level of intraline genetic homogeneity, whereas there is a considerable degree of interline genetic diversity. Since the interline variability of DTH reactions presents a continuous variation and is obviously larger than the intraline variability, we hypothesize that the DTH responsiveness is controlled by polygenic regulation. The challenge dose of SE also produces a small non-specific increase of the footpad thickness in non sensitized controls. An important finding of this experiment is that there is a positive correlation ($r = 0.55$, $P < 0.05$) between this non-specific inflammatory reaction and the intensity of the DTH of sensitized mice. It becomes there-

Table 1
Lack of correlation between the increase in footpad thickness and the number of splenic specific T cells in H and L mice immunized with SE.

Selection I	Number of SE-specific DTH T cells	Increase in footpad thickness 0.1 mm	Serum agglutinin titer	
			6th day	13th day
High	56,000/spleen	7.4	$<1/2$	1/500
———	$P<0.01$	n.s.	———	$P<0.001$
Low	5,000/spleen	8.1	$<1/2$	1/40

Footpad thickness was measured 24 h after s.c. challenge with 5×10^8 SE in cyclophosphamide treated mice (200 mg/kg) immunized 6 days previously by an i.v. injection of 10^8 SE. Specific T cells were enumerated in spleen 4 days after an i.v. injection of 10^9 SE.

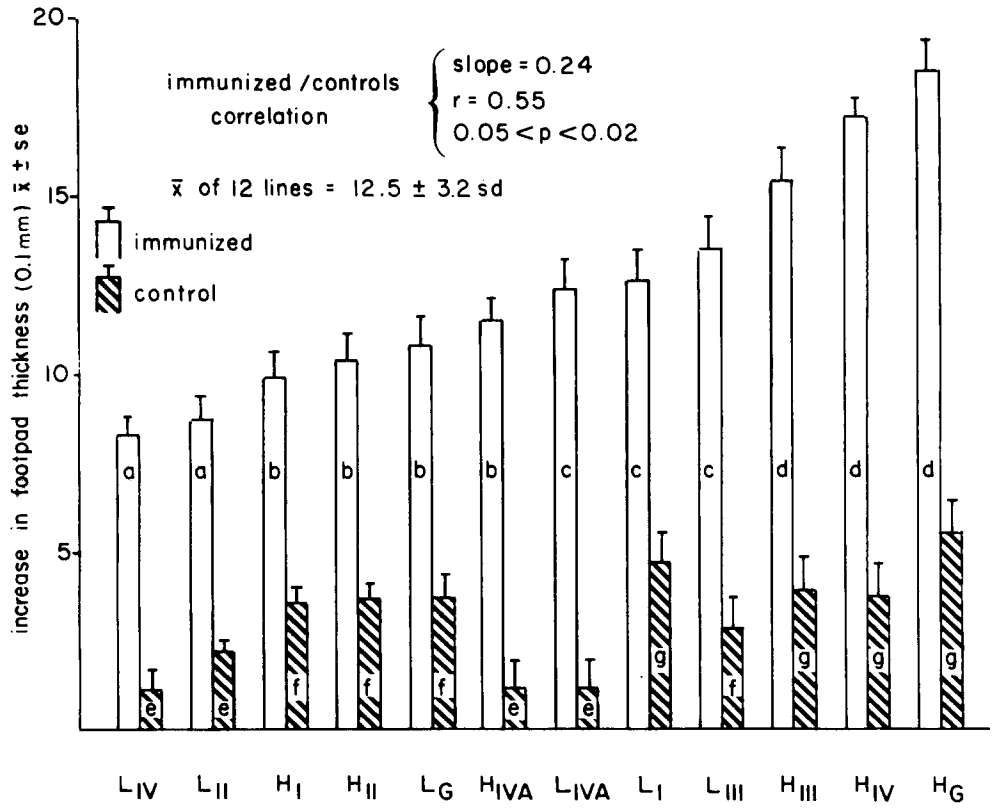


Fig. 5. Mean DTH response (± s.e.) in groups of H and L mice produced by 6 bidirectional selective breeding for antibody responsiveness. – Increase in left hind footpad thickness 24 h after challenge in cyclophosphamide (200 mg/kg) treated mice immunized with 10^8 SE in the right hind footpad and challenged 6 days later with the same dose of SE in the left hind footpad. The same dose of SE was injected in the footpad of non immunized mice. – Highly significant ($P<0.001$) differences between lines of mice are indicated by small letters ($a \neq b \neq c \neq d$) and ($e \neq f \neq g$) (From de Oliveira et al. 1985 [29]).

fore clear that the intensity of the specific DTH reaction does not correlate with the genetic status of specific immunity but rather with the postulated polygenic regulation of the non-specific inflammatory reactivity to a local irritant.

4. Genetic regulation of non-specific immunity

4.1. *Inflammatory reaction (IR)*

The beneficial role of inflammation against physical, chemical, infectious and tumoral aggressions has been taken into account since very ancient times, although it could also be held as a mixed blessing when the excessive intensity and extension of the phenomenon may be harmful to the whole organism [30–34]. Many publications deal with the physiopathology and biochemistry of inflammation; however, very little is known on the germ line genetic regulation of this important phenomenon. Few observations are reported on the different degree of some IR in distinct inbred animal lines [32]. Since the intensity of the IR, just like that of Ab responsiveness, is subject to polygenic control, the information obtained in inbred lines only gives a limited insight into the genetic regulation of IR, for the reasons already put forward concerning Ab responsiveness (see Fig. 1). Therefore, we have undertaken the quantitative study of the genetic regulation of the IR intensity.

To induce IR, we have used the model proposed by R. Fauve [35] based on the production of a local

IR by polyacrylamide microbeads (Biogel P-100, Bio-Rad, Touzard and Matignon, Paris) injected subcutaneously. This substance has the important advantage of being completely insoluble, totally chemically inert and non-antigenic. The intensity of the IR is therefore determined by a general host reactivity, irrespective of the nature of the irritant. For the study of IR in mice, we have standardized the following method: 0.75 ml of a 60% suspension of swollen Biogel in sterile apyrogen saline was injected subcutaneously into the shaved dorsal region. At different times after Biogel injection, the local exudate was collected in a plastic syringe through a large needle. The number of cells was counted and their morphology determined on May Grünwald-Giemsa stained smears. The protein concentration of the exudate was measured by ultraviolet spectrometry (280 nm) and expressed as Optical Density (O.D.). The kinetics of the IR in groups of random bred mice of both sexes is presented in Fig. 6. The different parameters of the IR increase very rapidly until a steady state period around the 3rd day. The score of cell concentration and O.D. on the 3rd day was therefore chosen to carry out further investigations. In Fig. 7 is represented the intensity of IR to Biogel P-100 measured in male mice of 14 lines produced in our laboratory by bidirectional selective breeding for immune response: Ab responsiveness (Selections I, II, III, IV, GP, GS) and for H and L "in vitro" T lymphocyte responsiveness to phytohemagglutinin [36, 37]. There is a marked difference in the IR between these mice populations. The cell concentration varied from $7.6 \pm 3.2 \times 10^6$ in L_{IV} to $88.7 \pm 24 \times 10^6$ in H_{II} with a continuous variation in IR intensity. The exudate protein concentration is positively correlated with the cell concentration. The least squares linear regression between these two parameters shows a very significant correlation coefficient (r). The IR intensity of the different populations investigated is completely independent of their H or L immunoresponsiveness status. For the reasons discussed above concerning the results in

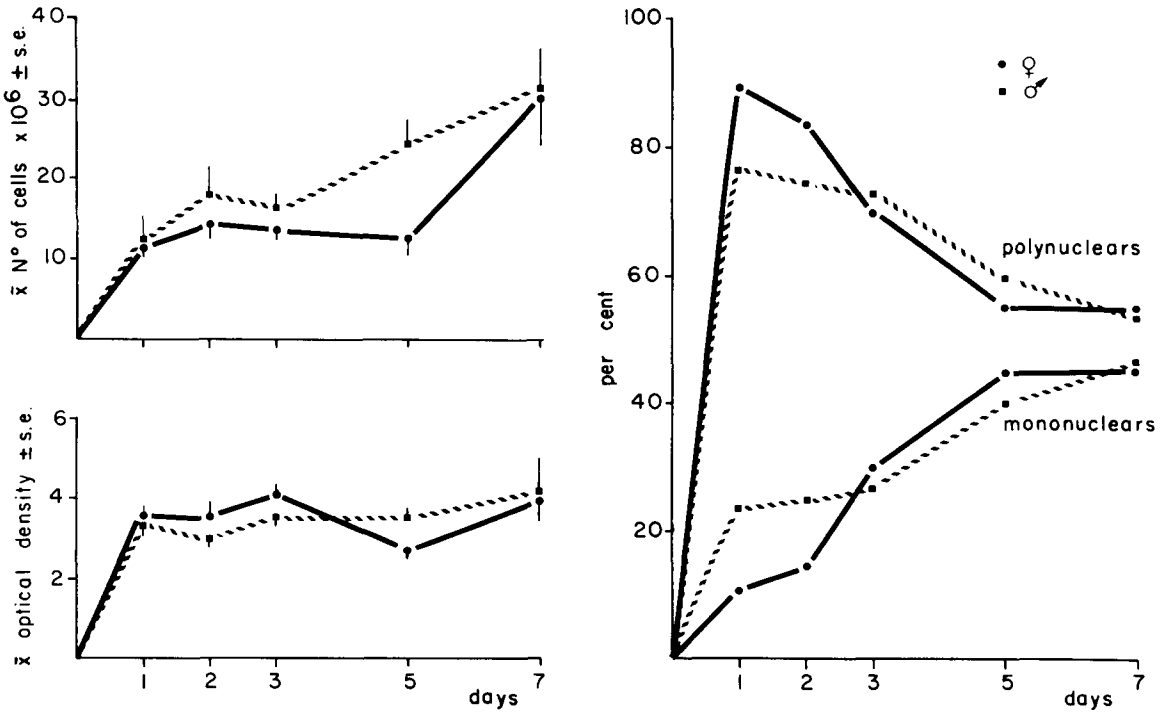


Fig. 6. Kinetics of IR in male and female random bred mice. Cell concentration (± s.e.), protein concentration (optical density at 280 nm) and percentage of mononuclear and polynuclear cells were determined in the exudate removed after dorsal subcutaneous injection of swollen Biogel P-100 (0.75 ml).

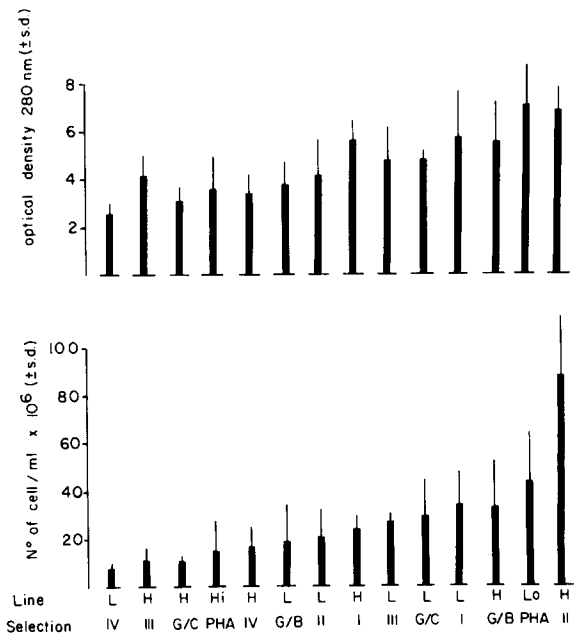


Fig. 7. Comparison of IR in male mice of H and L lines produced by 6 bidirectional selective breeding for antibody production (I, II, III, IV, GP and GS) and one selection for in vitro responsiveness to PHA (Hi and Lo). Cell concentration (± s.d.) and protein concentration (O.D. at 280 nm ± s.d.) in exudate removed 3 days after s.c. injection of 0.75 ml of Biogel P-100. Correlation coefficient r, between cell concentration and protein concentration = 0.87 ($P < 0.001$).

Fig. 5, the findings reported in Fig. 7 indicate that the IR intensity produced by Biogel P-100 is a quantitative character subject to polygenic regulation.

4.2. *Bi-directional selective breeding for maximum and minimum IR intensity*

The results presented above clearly show that the quantitative regulation of IR intensity is operated by the additive interaction of several alleles (polygenic regulation). The genetic analysis of a polygenic character is based on the production of lines of mice homozygous at the level of all the relevant alleles. These lines can only be obtained by bi-directional selective breeding protracted until Selection Limit.

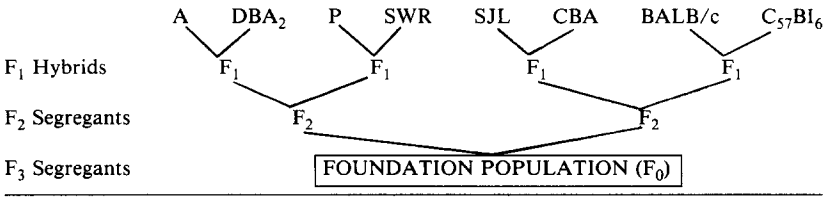
4.2.1. *Production of the foundation population (F_0)*

The initiation of a selective breeding experiment requires a highly genetically heterogeneous F_0 population. This was achieved by the balanced intercrossing of 8 inbred lines of mice chosen for their distinct origin as shown in Table 2. The IR intensity to Biogel P-100 measured in the 8 inbred lines chosen to produce the F_0 population is presented in Fig. 8. In terms of cell concentration, responsiveness is somewhat smaller in females than in males. Such a sex difference is not observed for the protein concentration. In both sexes, there is a significant positive correlation between mean values of cell number and protein concentration in the exudates. There is also a positive correlation between males and females data for each parameter. Both cell number and protein concentration present a continuous progressive variation in the two sexes, with a smaller intraline than interline variability. These results are consistent with those reported in Fig. 7 and confirm the conclusion that IR intensity to Biogel P-100 is a polygenic trait.

4.2.2. *Results of the first generation (F_1) of selective breeding for IR: heritability of the character*

The F_0 population consisted of 116 males and 103 females. Assortative matings of 18 pairs presenting

Table 2
Production of highly polymorph foundation population by equipoised crossing of 8 inbred lines chosen for their independent origin (Jackson Laboratory).



Each F_0 mouse contains a different recombination of 12.5% of the gene pool of the 8 inbred lines.

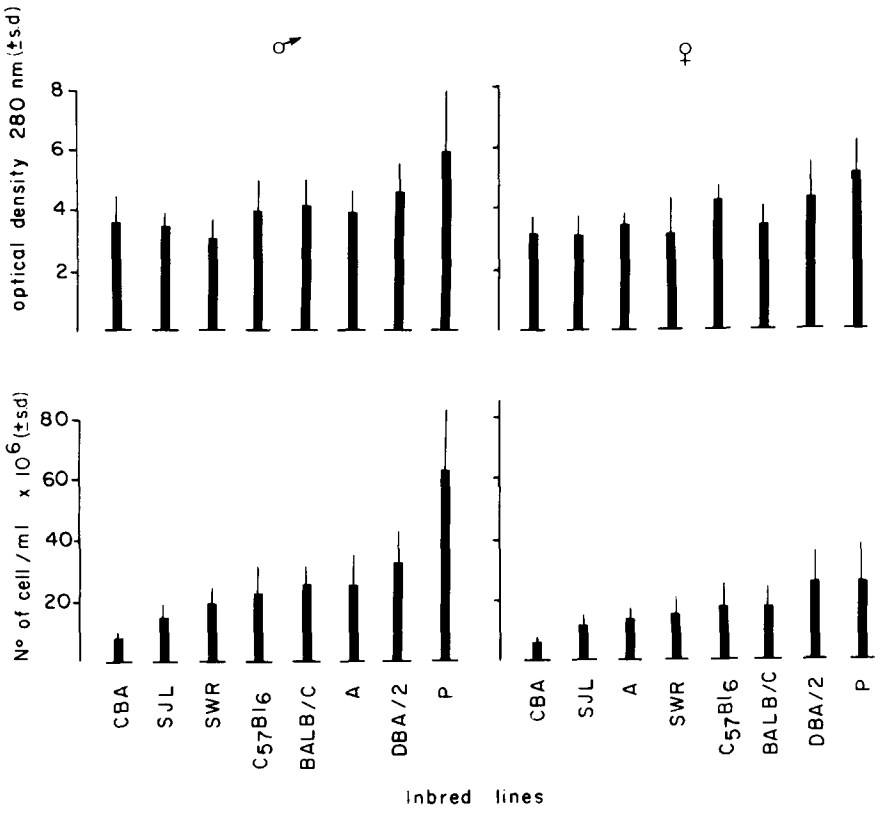


Fig. 8. Measure of IR in male and female mice of 8 inbred lines (From Jackson Laboratories). Same method as in Fig. 7. Correlation coefficient r: between cells and protein concentration = 0.81 in males and 0.84 in females; between ♂ and ♀ of the same inbred line = 0.87 for cell concentration and 0.92 for protein concentration.

the strongest IR and of 16 pairs presenting the weakest IR were performed to produce the F_1 generation of the two lines of the selective breeding. The results of IR intensity in the offsprings are presented in Table 3 as the values of the selection differential (S) and response to selection (R) calculated in terms of interline separation. The S value, which is the difference between the mean IR of the selected parents and that of F_0 population, is a measure of the selective genetic pressure resulting from the phenotypic assortative mating. The R value, which is the difference between the mean IR in F_1 and F_0 populations, measures the gain resulting from the selection. The ratio R/S which is the realized heritability (h_2) measures the fraction of the phenotypic paren-

Table 3
Heritability (h_2) of inflammatory reactivity to Biogel P-100 in the first generation of the selective breeding.

	Number of cells $\times 10^6$/ml		Protein concentration O.D.	
	♂	♀	♂	♀
Selection differential S	22.0	17.3	3.1	2.6
Response to selection R	2.8	3.4	0.7	0.6
h_2: $\frac{R}{S}$	13%	20%	22%	23%

Selection differential, S and response to selection, R are calculated from the results of interline divergence.

tal difference of the character which is actually inherited in the offsprings. This realized h_2 value, calculated in F_1 generations, is of the same order of magnitude as that observed in the five selections for Ab responsiveness (close to 20%) [3]. The h_2 value in this first generation is large enough to ensure the success of the IR selection which will be continued until selection limit in order to obtain "I Max" and "I Min" lines of mice.

5. Genetic regulation of specific immunoresponsiveness and anti-infectious immunity

The results in Section 3 concerning the genetic modifications of specific immunoresponsiveness produced in Selection I clearly demonstrate a causal opposition between lymphocyte and macrophage activity. L mice genetically endowed with high macrophage catabolic activity have a low level of specific responses, whereas the genetic deficiency of macrophage activity in H mice results in high specific immune responses.

Results on the outcome of carefully chosen experimental infections in the two lines of mice agree with their opposite status for these two important parameters of specific immunity. The infections reported in Table 4 have been chosen because it has been well established that either Ab responsiveness or macrophage activity is the principal defence mechanism. The infections listed in the left side of Table 4 are produced by the so called "extracellular parasites" which are sensitive to the defensive effect of Ab and C′. Against these infections, H line mice have a stronger natural resistance and are much more efficiently protected by specific vaccination than L line mice. The protective role of Ab against these infections can be demonstrated by the passive transmission of immunity to L line with H line specific immune serum.

The opposite outcome is observed for the infections produced by the so called "intracellular parasites" (right side of Table 4). These microorganisms, scarcely susceptible to the protective effect of Ab, are able to survive and multiply inside the phagocytic cells, mainly in macrophages. The bactericidal or bacteriostatic activity of macrophages constitutes then the principal defense mechanism. It is remarkable that L line is naturally more resistant than H line against these infections. Moreover the specific vaccination induces a high titer of specific serum Ab but has a low efficacy in H mice whereas L mice are efficiently protected in spite of weak antibody responses. The interline difference in resistance against the infections listed in Table 4 is extremely large because the Abs or the macrophages play the principal defensive role. In other infections where the importance of one or the other of these two mechanisms is not largely predominant, the interline difference in resistance/susceptibility is not so clear-cut.

On the basis of the experimental results on the outcome of several infections in Selection I, we have formulated a general theory accounting for several aspects of the anti-infectious immunity observed in natural populations of genetically heterogeneous individuals [1, 2, 4]. This theory, though convincing for some general aspects, is still incomplete, since it was formulated only on the basis of resistance mechanisms operated by the specific adaptive component of immunity. Obviously, non-specific, non-adaptive components of immunity, particu-

Table 4
Comparison of resistance to some infections in H and L lines in relation to the principal defense mechanism.

Antibody-mediated immunity HIGH more resistant than LOW	Macrophage-mediated immunity LOW more resistant than HIGH
Streptococcus pneumoniae	*Salmonella typhimurium*
Klebsiella pneumoniae	*Brucella suis*
Trypanosoma cruzi	*Yersinia pestis*
Plasmodia { *berghei*, *yoelii*, *chabaudi*	*Mycobacterium tuberculosis (BCG)*
	Leishmania tropica

larly the IR, play a very important defensive role too. We hope that the study of the resistance to experimental infections in I Max. and I Min. lines, when separated at Selection Limit, will contribute another batch of results which may be integrated into our theory in order to complete and extend its general meaning.

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